Veterinary Parasitology 169 (2010) 149–156

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Therapeutic and persistent efficacy of a long-acting (LA) formulation of ivermectin against *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) and sera concentration through time in treated cattle☆

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ARTICLE INFO

Article history:
Received 31 August 2009
Received in revised form 15 December 2009
Accepted 30 December 2009

Keywords:
Rhipicephalus (*Boophilus*) *microplus*
Ivermectin
Control
Cattle fever tick
Endectocides
Macrocyclic lactones

ABSTRACT

The concentration–time profile, therapeutic, and persistent efficacy of a single subcutaneous injection of cattle with a long-acting (LA) formulation of ivermectin at a concentration of 630 μg/kg of body weight were determined against *Rhipicephalus* (*Boophilus*) *microplus*. Ivermectin sera concentration in treated cattle increased to 13.0 ppb within 1 d after treatment, and peaked at 26.2 ppb at 11 d post-treatment. Ivermectin sera levels remained above the threshold level for control of feeding ticks (≥8 ppb) for 42.6 d after treatment. Therapeutic efficacy of ticks on treated animals was >99.9%, and tick number, index of fecundity, engorgement weight, and egg mass weight of ticks from treated animals remained dramatically less than ticks from untreated animals. Tick number and reproductive capacity of ticks infested on treated animals at 14 and 28 d post-treatment were less than for ticks on untreated animals, whereas engorgement weight and egg mass weight of treated ticks remained lower than that of untreated ticks 49 d post-treatment. However, the level of control against ticks infested at 14 d after treatment (99.9%) was the only post-treatment infestation interval that provided the required 99% control necessary for use in the U.S. tick eradication program. The 14 d post-treatment infestation was also the only interval at which infested ticks were exposed to ivermectin levels above the threshold level of 8 ppb for the entire parasitic development period. Cattle would have to be treated at intervals of no more than 31 d apart to ensure that no viable ticks could reach repletion and detach from the host. Although this treatment interval is >2-fold longer than the present treatment requirement (14 d), it is dramatically less than the label claim for the LA ivermectin formulation of 75 d of prevention against re-infestation.

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1. Introduction

The federally managed Cattle Fever Tick Eradication Program (CFTEP) is responsible for preventing the re-establishment and dispersal of cattle fever ticks (CFT), *Rhipicephalus* (*Boophilus*) spp. within the U.S. by placing infested premises under quarantine, thus preventing or strictly regulating movement of cattle. The owner of quarantined premises must choose one of the two options listed in Title 4, Part 2, Chapter 41 of the Texas Administrative Code to comply with quarantine regulations. The first

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0304-4017/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetpar.2009.12.040

option (Rule 41.8-Dipping and Treatment of Livestock), if the owner wishes to maintain cattle in the premises, requires all cattle (100%) to be gathered and dipped in the organophosphate (OP) acaricide, coumaphos every 14 d for a period of 6–9 months, depending on the time of year the infestation is discovered. Thus, the cattle must be treated a total of 14–21 times before the quarantine is lifted. The second option (Rule 41.9-Vacation and Inspection of a Premise) called "pasture vacation," if the owner does not wish to gather and dip cattle every 14 d, requires dipping the cattle until free of ticks then the removal of all livestock (100%) for the same 6–9-month period as the first option. The objective of the "pasture vacation" option is to ensure that all free-living ticks on the premises will die from starvation or desiccation during the quarantine period due to a lack of suitable hosts upon which to feed.

The option of maintaining livestock on infested premises places a heavy financial burden on the owner/producer because it is their responsibility to bear the costs associated with gathering and handling the cattle every 14 d during the quarantine period. Therefore, "pasture vacation" has historically been the most frequently chosen option, since gathering/handling costs are minimized. Unfortunately, in recent years the "pasture vacation" option has, upon several occasions, failed to eliminate CFT infestations within the duration of the quarantine period, and the incidence of these failures appears to be increasing. These failures have largely been attributed to the enormous rise in numbers of white-tailed deer, *Odocoileus virginianus* (Zimmermann) in Texas, to an estimated 3.1 million animals by 1991 (Texas Tech University, 1997). Although deer are not nearly as suitable hosts of CFT as are cattle (Cooksey et al., 1989; Davey, 1990), they can easily sustain the tick population long enough to surpass the length of the quarantine period, especially when the population density is high at the onset of the quarantine period. Consequently, the risk of deer becoming involved in the host–parasite cycle, thereby sustaining and/or dispersing ticks to un-infested areas during the quarantine period has, in recent years, led CFTEP officials to encourage producers/owners to maintain, gather, and dip cattle during the quarantine period rather than vacate the infested premises. However, it has been difficult to convince producers/owners to adopt the dipping option instead of the "pasture vacation" option because of the high cost of handling and dipping animals on a frequent basis. Thus, there is a critical need for the development of an acaricide treatment strategy that would substantially reduce the number of gatherings/dippings necessary, while still achieving eradication, and provide owners/producers with the incentive to maintain cattle on infested premises during the quarantine duration rather than vacating the premises.

Currently organophosphate (OP), pyrethroid (P), formamidine (FORM), and macrocyclic lactone (ML) chemicals are the major classes of acaricides used in the U.S. and/or Mexico for controlling CFT. However, one important factor associated with the use of these agents that has made U.S. producers/owners reticent to choose the dipping option instead of the "pasture vacation" option is that, while the acaricides are all highly effective against ticks that are feeding on the hosts at the time of treatment (Davey and Ahrens, 1982; Ahrens et al., 1989, 1998; Davey and George, 1998; George et al., 1998; George and Davey, 2004), the residual effectiveness (protective period against re-infestation) of all of them is rather short, thereby requiring frequent treatments to ensure eradication. The OP acaricide, coumaphos, which has been used in the U.S. CFTEP almost exclusively since 1968 (Graham and Hourrigan, 1977), has a reported protective period against larval re-infestation of $\leq$8–9 d (Wharton et al., 1970; Davey et al., 1983; Singh and Chhabra, 1992). Similarly, the protective period of P acaricides, such as permethrin, cypermethrin, and decamethrin ranges from 1.1 to 15 d (Nolan et al., 1979; Khan and Srivastava, 1988) and 7–10 d for the FORM, amitraz (Roy-Smith, 1975; Davey et al., 1984).

Currently in Mexico ML endectocides, such as ivermectin, doramectin, and moxidectin, are being used more frequently than ever before. Although endectocides provide excellent control ($\geq$99%) at low concentrations against ticks on the host at the time of treatment (George and Davey, 2004; Davey et al., 2005), they are less effective against ticks in the final stages of engorgement when treated (Davey and George, 2002; Davey et al., 2007). In addition, the residual activity (protection against re-infestation) of many ML acaricides is not dramatically longer than OP, P, and FORM acaricides (George and Davey, 2004; Davey et al., 2005). Thus, it appears that the use of ML acaricides would require that treatment intervals be rather short ($\leq$14 d) to ensure that no ticks could reach repletion between repeated treatments. However, in recent years the development of long-acting (LA) formulations of some ML acaricides have become commercially available in other countries outside of the U.S., and one which is registered in Mexico claims up to 75 d of protection following treatment. If the claims on these materials are true, then these compounds could benefit the U.S. CFTEP enormously.

The objective of this study was to evaluate the residual efficacy (duration of protection against re-infestation) of a LA formulation of ivermectin against larvae of *R.* (*B.*) *microplus* released on cattle at various intervals following treatment. Results obtained from this study could provide the CFTEP with an alternative treatment strategy that would reduce the number of treatments necessary to eliminate cattle fever ticks from an infested premise. In addition, positive results could provide the necessary incentive for owners/producers to maintain cattle on the infested premise during the quarantine period rather than choosing the "pasture vacation" option as the means of eliminating the tick population.

2. Materials and methods

The study was conducted at the USDA, Agricultural Research Service (ARS), Cattle Fever Tick Research Laboratory (CFTRL), in Edinburg, TX, which is a federally approved quarantined facility authorized to conduct research studies on *Rhipicephalus* (*Boophilus*) ticks. Twelve Hereford calves, each weighing ca. 200 kg and with no prior exposure to *Rhipicephalus* (*Boophilus*) ticks, were randomly divided into two groups of six animals per group. One

group of six calves designated as an untreated group served as a negative control group to which the treated group was compared. The second group of calves was treated with the test agent at the manufacturer's recommended dosage according to body weight. The acaricide, Ivomec GOLD® (Merial Inc., Mexico, Marques, Qro., Mexico) is registered for use in Mexico on cattle for control of internal and external parasites. The product was obtained commercially over-the-counter in Mexico and was declared and passed through U.S. Customs into the U.S. and brought to the CFTRL for testing. The material (Ivomec GOLD®) was an injectable formulation containing 3.15% active ingredient (AI) of ivermectin with a recommended dosage rate of 1 ml per 50 kg of body weight, which produced a concentration of 630 μg of ivermectin per kg of body. All calves were weighed on certified scales at ≤1 week before application of the test material to ensure proper dosing. Throughout the study all animals were held in an open-sided barn under ambient conditions, except that a roof prevented direct sunlight or rainfall from reaching the animals. During the study each animal was held in an individual stanchion within a 3.3 m × 3.3 m stall, and stalls were separated from each other by 1.7 m high cinder block walls, which prevented crossover of ticks between animals.

2.1. Therapeutic efficacy

On three separate occasions, at −18, −11, and −4 d before treatment, all calves (both groups) were artificially infested with approximately 5000 larval *R.* (*B.*) *microplus* ticks (as determined by egg weight, i.e. 250 mg of eggs, ca. 5000 larvae) that were 2–4 weeks old. A 16 mm × 70 mm shell vial (2-dram) containing the larvae was glued to the midline of the back of each animal with branding cement and the cotton plug removed to allow the larvae to disperse over the body of the animal. This pretreatment infestation pattern allowed for evaluation of the Ivomec GOLD® against all parasitic development stages of the tick (larva, nymph, and adult) on the host at the time of the treatment. On the day of treatment (Day 0), each treated calf was injected subcutaneously with the test material at the manufacturer's recommended concentration according to body weight. Beginning on the day after treatment (Day 1) and continuing through Day 34 post-treatment, engorged female ticks that had detached from each animal were collected from the floor of the stall and counted and recorded daily. A random sample of up to 10 engorged females (whenever possible) was saved from each animal on each day of the evaluation period (Day 1 through Day 34 post-treatment) to obtain oviposition and fertility data. Tick samples obtained from each animal each day were weighed collectively, placed in a coded 9-cm diameter plastic Petri dish, and held in an incubator at 27 ± 2 °C, 92.5% RH, under a 12:12 L:D cycle for 20 d. After 20 d, eggs from each individual sample were harvested, weighed, placed in a coded shell vial, and returned to the incubator. Spent females were discarded. At 4 weeks after eggs were weighed, the hatch rate of each sample was visually estimated by comparing the proportion of unhatched eggs to the proportion of egg shells present in the vial, as described by Davey et al. (2005). Data for tick counts, egg mass weights, and egg hatch for each animal over the entire 34-d post-treatment evaluation period were used to calculate the Index of Fecundity (IF) of each animal on each day using the formula reported by Davey et al. (2001): IF = No. of ♀♀ collected × (Wt. of eggs (g)/No. of ♀♀ saved) × egg hatch (%).

Calculation of IF provided a method of estimating the reproductive capacity of the ticks recovered from each calf on each of the 34 d following treatment. The percentage control was determined by comparing ticks from treated calves with data obtained from ticks from untreated calves using the following modified Abbott's (1925) formula: % control = (mean IF of untreated − summed IF of each treated calf/mean IF of untreated) × 100.

In addition to calculated IF values, the biological data (female weight and egg mass weight) of sampled ticks from the untreated group were compared to data obtained from treated calves to determine any measurable effect on weight and fecundity of females that survived the treatment.

2.2. Persistent efficacy (determination of protective period against larval re-infestation)

The same cattle used in the therapeutic portion of the study were used to evaluate the persistent efficacy (protective period against re-infestation) of the test material. Determination of the duration of the protective period against larval re-infestation following treatment was assessed based on a series of larval infestations that were applied to all cattle (both groups) at various intervals following treatment. To determine the persistent efficacy at 14 d (2 weeks) post-treatment each calf was artificially infested with ca. 2500 larval *R.* (*B.*) *microplus* ticks, as in the therapeutic trial, by attaching a 17 mm × 60 mm (2-dram) shell vial containing the larvae to the midline of the back of each animal and removing the cotton stopper. To assess the protective period (persistent efficacy) at 28, 35, 42, 49, 56, 63, and 70 d (4–10 weeks) post-treatment, all calves were infested with ca. 2500 larvae at each interval, as described above. A system based on the detachment pattern of *R.* (*B.*) *microplus* reported by Hitchcock (1955), which showed that ≥95% of a cohort of ticks infested at the same time will detach from the animal at 21–27 d after infestation, was used to establish the week when engorged females had been placed on the animal as larvae (Table 1). The protective period (persistent efficacy) was based on tick counts, fecundity, and fertility data collected at 21–27 d following each post-treatment infestation. Beginning at 35 d post-treatment and continuing through 97 d post-treatment, daily tick collection and sampling of each animal was conducted as described in the therapeutic efficacy portion of the study. Daily IF of each calf on each day between 35 and 97 d following treatment was calculated using the previously described formula. To determine the persistent (residual) efficacy at each post-treatment infestation interval, the mean IF value of the untreated control group at each post-treatment classification interval was compared to the summed IF value of each calf in the treated group having the same post-treatment classification interval, using the previously described

Table 1
Relationship between post-treatment larval infestation and subsequent collection of detached engorged females recovered at 21–27 d after infestation.

Days post-treatment of larval infestation	Days post-treatment at which detaching engorged females correspond to 21–27 d after infestation at the indicated larval infestation interval
14	35–41
28	49–55
35	56–62
42	63–69
49	70–76
56	77–83
63	84–90
70	91–97

formula, thus facilitating statistical analysis of the data. In addition to calculating IF value and percentage control level at each post-treatment infestation interval, biological data (female engorgement weight and egg mass weight) obtained from the sampled females collected from both untreated and treated calves at each classification interval were compared to determine any measurable adverse effect in female weight and fecundity through time that could be attributed to the treatment.

2.3. Pharmacokinetics evaluation

Following the tick control evaluation portion of the study, the treated calves were placed in an un-infested area for 3 weeks after which blood samples were obtained and analyzed to ensure that there was no ivermectin in the blood system of any of the calves. Then, the cattle were re-weighed and re-treated with the test material at the recommended dosage (by animal weight) and allowed to roam freely in a 7.2 ha un-infested pasture. This was done so that the concentration of the test material in the blood serum of treated animals could be determined at various post-treatment intervals under natural field conditions. Using 12.5 ml SST Vacutainers (Tyco Healthcare Group LP, Mansfield, MA) blood samples were taken at 1, 4, 7, 11, 14, 17, 21, 24, 28, 35, 42, 49, 56, 63 and 70 d post-treatment from the jugular vein of each animal. Blood was centrifuged at 2000 rpm for 30 min to obtain serum, which was poured into individually coded 14 ml polypropylene round bottom tubes (Becton Dickinson, Franklin Lakes, NJ) and frozen at −80 °C for later analysis. At the time of analysis, sera samples were thawed and placed in an HPLC to determine concentration of the test material in the serum, as reported by Oehler and Miller (1989). The technique enables quantification ≥2 ppb of the test material in 5 ml of serum.

2.4. Data analysis

Data on tick number, IFF, female weight, and egg mass weight obtained for untreated and treated females in the therapeutic portion of the study (ticks on the host at the time of treatment) were analyzed by Mann–Whitney Rank Sum Test to determine differences for each measured parameter (Systat Software, 2006). Data from the persistent efficacy portion of the study were analyzed by two methods. First, the tick number, IF, engorgement weight, and egg mass weight value for untreated and treated ticks at each post-treatment infestation interval were subjected to either a *t*-test or Mann–Whitney Rank Sum Test to determine differences between the two groups. The tick number, IF, engorgement weight, egg mass weight, and percentage control of treated ticks were then analyzed by repeated measures analysis of variance (RM-ANOVA) across all post-treatment infestation intervals and differences among means were determined either by Holm–Sidak or Kruskil–Wallis methods (Systat Software, 2006).

3. Results

3.1. Pharmacokinetics evaluation

Ivermectin was absorbed into the blood of treated cattle very quickly after treatment, reaching 13.0 ± 2.1 ppb the day after treatment (Fig. 1). Maximum ivermectin concentration occurred 11 d post-treatment at 26.2 ± 4.7 ppb, and at 4 and 7 d post-treatment was only slightly lower (25.7 ± 6.8 and 24.9 ± 6.5 ppb, respectively). Ivermectin concentration decreased 14–42 d post-treatment, from 21.6 ± 3.2 ppb at 17 d post-treatment to 8.6 ± 2.1 ppb at 42 d post-treatment. From 50 to 70 d post-treatment mean ivermectin concentration remained ≤6.0 ppb in all samples, and several individual samples produced undetectable levels (0.0 ppb).

3.2. Therapeutic efficacy

Significantly fewer ($T = 57.0$; $df = 6, 6$; $P < 0.003$) ticks released on cattle before treatment reached repletion on treated cattle than on untreated cattle (Table 2). Similarly, the reproductive capacity (IF) of females that survived to repletion on treated cattle was also significantly lower ($T = 57.0$; $df = 6, 6$; $P < 0.003$) than that of untreated ticks, resulting in >99.9% control. Engorgement weight of females recovered from treated animals was significantly less ($T = 139.0$; $df = 16, 171$; $P < 0.001$) than untreated females, as was the mean weight of egg masses produced by treated females ($T = 208.0$; $df = 16, 171$; $P < 0.001$).

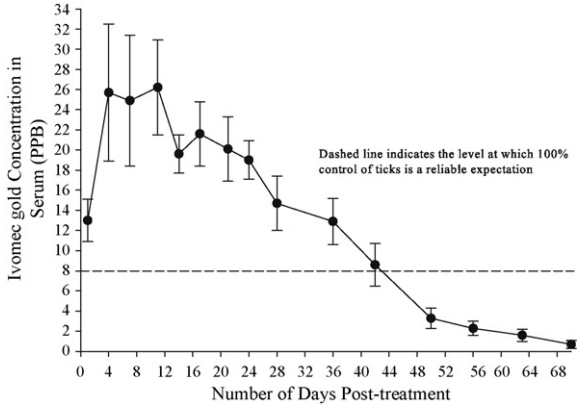

Fig. 1. Mean ± SE concentration of ivermectin in serum of cattle treated with a single subcutaneous injection of a long-acting formulation of ivermectin at 630 μg/kg body weight.

Table 2
Mean ± SE tick number per calf, female weight, egg mass weight, index of fecundity and fertility (IF), and percentage control of *Rhipicephalus* (*Boophilus*) *microplus* recovered from untreated and treated cattle infested at 18, 11, and 4 d prior to a single subcutaneous injection of a long-acting (LA) ivermectin formulation at 630 μg/kg body weight.

Treatment	Number of ticks per calf	Female weight (mg)	Egg mass weight (mg)	IF	Percentage control
Untreated	2274 ± 97 a	344 ± 5 a	110 ± 4 a	246.103 ± 24.438 a	–
Treated	5 ± 3 b	38 ± 9 b	1 ± 1 b	0.006 ± 0.006 b	>99.9 ± 0.0

Means tested by Mann–Whitney Rank Sum Test; means within the same column followed by a different letter are significantly different ($P < 0.05$). Number of ticks per calf, $T = 57.0$; $df = 6, 6$; $P = 0.002$; female weight, $T = 139.0$; $df = 16, 171$; $P < 0.001$; egg mass weight, $T = 208.0$; $df = 16, 171$; $P < 0.001$; IF, $T = 57.0$; $df = 6, 6$; $P = 0.002$.

3.3. Persistent efficacy (determination of protective period against larval re-infestation)

Comparison between untreated and treated animals at each post-treatment larval infestation interval showed that larvae released at 14 and 28 d post-treatment produced significantly fewer engorged females per animal with a significantly lower reproductive capacity (IF) ($P < 0.05$) than that of the untreated group (Table 3). Females that developed from larvae released 35–70 d post-treatment did not differ significantly ($P > 0.05$) in the number of ticks per animal or the reproductive capacity (IF) between the untreated and treated groups, even though the untreated group generally produced more ticks per animal with a higher IF value at each interval, except for larvae infested at 70 d post-treatment.

Number of ticks per animal across all infestation intervals for treated cattle differed significantly ($F = 7.76$; $df = 7, 35$; $P < 0.001$) among infestation intervals (Table 3). The number of engorged females per animal that developed from larvae released 14 d post-treatment was significantly lower ($P < 0.05$) than at all other infestation intervals, except for the larvae released at 28 d post-treatment, even though the number of ticks per animal at 28 d was considerably greater than those infested at 14 d post-treatment. Fewer ticks were recovered from the 28 d post-treatment interval ($P < 0.05$) than from larvae released at 42 d, but otherwise there was no difference ($P > 0.05$) in number of ticks per animal at any interval after the 28 d post-treatment infestation.

IF (reproductive capacity) of ticks across all post-treatment infestations for treated cattle also differed significantly ($F = 25.0$; $df = 7, 35$; $P < 0.001$) among infestation intervals (Table 3). Reproductive capacity of engorged females developed from larvae infested 14 d post-treatment was significantly lower ($P < 0.05$) than that of females recovered at all other post-infestation intervals. While the IF value of ticks developed from larvae infested at 28 d post-treatment was lower ($P < 0.05$) than that of ticks infested at 70 d post-treatment, reproductive capacity was not different ($P > 0.05$) from ticks infested at the remaining intervals. Ticks released 35–70 d post-treatment showed no difference ($P > 0.05$) in IF.

The percentage control across all post-treatment infestation intervals showed a progressive and significant decrease through time ($F = 11.2$; $df = 7, 35$; $P < 0.001$) (Table 3). The percentage control achieved against ticks infested at 14 d post-treatment was greater ($P < 0.05$) than all other intervals, except ticks infested at 28 d post-treatment, even though control against ticks infested at 28 d after treatment (70.4%) was 20.5% lower than the 99.9% level of control obtained ticks infested at 14 d post-treatment. Greater control ($P < 0.05$) was achieved against ticks released at 28, 42, and 49 d post-treatment than was obtained against ticks released at 70 d post-treatment, but did not differ from infestations applied at 35, 56, and 63 d post-treatment, which did not differ from each other or from ticks released at 70 d post-treatment.

Female engorgement weight and egg mass weight of ticks from treated animals from infestations applied at

Table 3
Mean ± SE number of ticks per calf, index of fecundity and fertility (IF), and percentage control of *Rhipicephalus* (*Boophilus*) *microplus* females that survived to repletion from larval infestations applied to untreated and treated cattle at various intervals following a single subcutaneous injection of a long-acting (LA) ivermectin formulation at 630 μg/kg of body weight.

Days post-treat larvae infested	Number of ticks per animal			Index of fecundity			Percentage control
	Untreated	Treated	Analysis between treatment	Untreated	Treated	Analysis between treatment	
14	618 ± 60	1 ± 1 a	*	58.2 ± 8.6	0.1 ± 0.1 a	*	99.9 ± 0.1 a
28	475 ± 55	169 ± 72 ab	*	38.4 ± 6.7	11.4 ± 5.4 b	*	70.4 ± 14.1 ab
35	500 ± 60	421 ± 115 bc	NS	57.1 ± 9.7	34.1 ± 13.0 bc	NS	46.5 ± 18.9 bc
42	555 ± 80	472 ± 87 c	NS	62.1 ± 13.5	28.3 ± 8.6 bc	NS	54.6 ± 13.7 b
49	225 ± 52	255 ± 53 bc	NS	21.7 ± 7.7	10.1 ± 2.4 bc	NS	53.6 ± 11.0 b
56	519 ± 109	408 ± 65 bc	NS	49.4 ± 12.4	34.2 ± 5.7 bc	NS	32.0 ± 10.8 bc
63	459 ± 92	414 ± 52 bc	NS	43.8 ± 13.6	30.4 ± 4.6 bc	NS	30.6 ± 10.5 bc
70	322 ± 52	411 ± 44 bc	NS	30.2 ± 7.1	42.1 ± 7.4 c	NS	7.4 ± 5.4 c

Means in each row for number of ticks per calf and IF were tested by *t*-test or Mann–Whitney Rank Sum Test ($P < 0.05$); each row of columns 4 and 7 indicates difference between untreated and treated cattle for each parameter; asterisk indicates significant difference, while NS indicates no difference. Means within columns 3, 6, and 8 were tested by repeated measures (RM) analysis of variance (ANOVA); differences among means over all post-treatment larval infestation intervals were determined by the Holm–Sidak or Kruskil–Wallis method ($P < 0.05$).

Table 4
Mean ± SE female engorgement weight and egg mass weight of *Rhipicephalus* (*Boophilus*) *microplus* females that survived to repletion from larval infestations applied to untreated and treated cattle at various intervals following a single subcutaneous injection of a long-acting (LA) ivermectin formulation at 630 μg/kg of body weight.

Days post-treat larvae infested	Female engorgement weight (mg)			Egg mass weight (mg)		
	Untreated	Treated	Analysis between treatment	Untreated	Treated	Analysis between treatment
14	313 ± 9	123 ± 31 a	*	98 ± 6	13 ± 13 a	*
28	360 ± 10	201 ± 14 ab	*	100 ± 7	54 ± 8 a	*
35	321 ± 11	205 ± 14 ab	*	105 ± 8	70 ± 7 ab	*
42	321 ± 10	228 ± 14 ab	*	110 ± 6	64 ± 7 ab	*
49	291 ± 17	218 ± 13 ab	*	98 ± 8	63 ± 6 ab	*
56	278 ± 12	252 ± 12 bc	NS	86 ± 7	89 ± 7 bc	NS
63	280 ± 11	269 ± 11 c	NS	81 ± 8	81 ± 6 abc	NS
70	281 ± 11	275 ± 11 c	NS	92 ± 7	98 ± 7 c	NS

Means in each row for each measured parameter were tested by *t*-test or Mann–Whitney Rank Sum Test ($P < 0.05$); each row of columns 4 and 7 indicates difference between untreated and treated cattle for each parameter; asterisk indicates significant difference, while NS indicates no difference. Means within columns 3 and 6 were tested by repeated measures (RM) analysis of variance (ANOVA); differences among means over all post-treatment larval infestation intervals were determined by the Holm–Sidak or Kruskil–Wallis method ($P < 0.05$).

14–49 d post-treatment were significantly lower ($P < 0.05$) than engorgement weights and egg mass weights for females from untreated calves for each respective infestation (Table 4). Conversely, there was no difference ($P > 0.05$) in engorgement weight of untreated and treated females recovered from infestations applied at 56–70 d post-treatment, even though mean weight of untreated ticks was greater at each interval. Likewise, there was no difference ($P > 0.05$) in the mean weight of egg masses produced by untreated and treated females obtained from infestations applied at 56–70 d post-treatment, but egg masses of treated females actually weighed as much or slightly more than those of untreated females.

Female engorgement weight of ticks from treated cattle across all post-treatment infestations differed significantly ($F = 9.84$; $df = 7, 223$; $P < 0.001$) among the infestation intervals (Table 4). While engorgement weight of females obtained from ticks infested at 14 d post-treatment was lower than all other infestation intervals, there was no difference ($P > 0.05$) from that of larvae infested at 28–49 d post-treatment. Although female engorgement weight at 70 d post-treatment was greater than all other intervals, there was no difference ($P > 0.05$) from weights of ticks released at 56 or 63 d post-treatment.

Mean egg mass weight of females obtained from treated cattle across all post-treatment infestation intervals showed a similar trend to that of engorgement weight, producing significant differences ($F = 6.23$; $df = 7, 223$; $P < 0.001$) among the various infestation intervals (Table 4). Again mean egg mass weight of females that developed from larvae infested at 14 d post-treatment was lower than all other intervals, but was not different ($P > 0.05$) from ticks infested at 28–49 d post-treatment. Likewise, egg mass weight derived from ticks infested at 70 d post-treatment was greater than all other intervals, but was not different ($P > 0.05$) from that of ticks released at 56 or 63 d post-treatment.

4. Discussion

These results demonstrated that the therapeutic efficacy of the LA ivermectin formulation provided virtually complete control (>99.9%) against ticks in all parasitic development stages on the host at the time of treatment. This therapeutic level of control was not surprising considering the treatment concentration of 630 μg/kg of body weight was slightly greater than 3 times the concentration recommended for the traditional injectable formulation of ivermectin (200 μg/kg of body weight), which has been reported to provide ≥99.0% control of all parasitic stages of *R.* (*B.*) *microplus* infested on host animals at the time of treatment (Maske et al., 1992; Davey et al., 2005). Even though the level of control of both the traditional and LA formulations were both equal to or above the standard 99% considered to be the minimum acceptable level for use in the U.S. CFTEP, the LA formulation allowed only one fifth as many ticks per animal (5 ± 3 ticks) to reach repletion as the 98 ± 76 ticks reported for the traditional injectable formulation using the same larval infestation rate (Davey et al., 2005). Thus, the use of the LA formulation would provide a distinct advantage in the CFTEP, where assessment of eradication is predicated merely on the presence or absence of ticks on the host animals.

Even though some investigators have reported that the concentration of endectocide in sera of cattle that provides antiparasitic activity is unclear (Lifschitz et al., 2007; Toutain et al., 1997), others have reported a level of 5–8 ppb of an endectocide in the sera of cattle to be the threshold level at which control of feeding ticks can be expected (Nolan et al., 1985; Pound et al., 1996; Miller et al., 1999). Results of this study indicated ivermectin levels in the sera of treated cattle increased quickly after treatment, reaching ≥24.9 ± 6.5 ppb at 4–11 d after treatment, with peak concentration (26.2 ± 4.7 ppb) occurring at 11 d post-treatment (Fig. 1). Furthermore, mean ivermectin concentration remained ≥8.6 ± 2.1 ppb for 42 d following treatment. These results were strikingly similar to a

pharmacokinetics study (Lifschitz et al., 2007) conducted using the same LA formulation of ivermectin used in this study, which reported a virtually identical concentration–time profile of ivermectin in cattle sera with a maximum ivermectin concentration of 26.0 ± 3.55 ppb and peak concentration occurring 9.14 ± 2.87 d after treatment. In contrast to this study, pharmacokinetic data of the traditional 1% injectable formulation of ivermectin in cattle treated at a concentration of 200 μg/kg of body weight showed that sera concentration decreased below the 8 ppb threshold level 2.8 times sooner (ca. 15 d post-treatment), with a maximum concentration that was 17.4% higher (31.7 ppb), which occurred 2.75 times earlier (3.98 d post-treatment) (Toutain et al., 1997).

Development of the LA formulation of ivermectin was predicated on the perceived need for an extended period of protection against re-infestation as a means of reducing costs incurred by producers necessitated by frequent treatments (Lifschitz et al., 2007). These results demonstrated that in comparison to ticks recovered from untreated cattle, tick numbers and reproductive capacity (IF) of ticks from treated cattle were significantly reduced when ticks were infested at 14 and 28 d post-treatment, while engorgement weights and egg mass weights were adversely affected in ticks infested up to 49 d post-treatment. However, even though adverse effects were observed in treated ticks infested at intervals of 28–49 d post-treatment, the duration of the protective period against larval re-infestation, during which the level of control was ≥99%, was achieved only against ticks infested at 14 d post-treatment. Thus, against ticks infested at 28–70 d after treatment, the level of control was well below that considered acceptable for use in the CFTEP (≥99%), and far below the label claim of 75-d prevention of re-infestation for the Ivomec GOLD® registered for use in Mexico.

Comparison of the percentage control data with the concentration–time profile of ivermectin in sera of treated cattle indicated that a level of ≥99% control could be expected only when ticks were exposed to the threshold concentration of ≥8 ppb of ivermectin during the entire parasitic development period (21–27 d), as was the case in ticks infested at 14 d post-treatment. By contrast, the resulting level of control decreased to 70.4% against ticks exposed to the threshold ivermectin concentration (≥8 ppb) for 12 d of the parasitic development period, as was the case for ticks infested at 28 d post-treatment. Similarly, the level of control dropped to 46.5% against ticks exposed to the threshold ivermectin level for only 7 d of the parasitic development period, as was the case for ticks infested at 35 d post-treatment. Thus, while Toutain et al. (1997) reported that it was unclear whether duration of drug exposure was important for antiparasitic activity, these data indicated that the length of drug exposure at the threshold dose (≥8 ppm) during the parasitic development of the ticks was an important factor in the subsequent level of control that could be expected, i.e. the longer the exposure at or above the threshold concentration the greater the degree of control that resulted. Using the same LA ivermectin formulation against *R.* (*B.*) *microplus*, Borges et al. (2008) reported a protective period against larval re-infestation of >99% that was about 21 d longer than results obtained in this study, and efficacy levels at each comparable infestation interval were 20–30% greater. In even greater contrast to our results, another study (Carvalho et al., 1999) conducted with LA formulations, which included the same formulation used in the present study, showed reductions of >90% occurred in tick counts and reproductive capacity for 116–129 d following a single treatment. While these studies suggested a longer persistent activity than the present study, the reason may be due, in part, to the fact that in other countries claims for control of ticks are predicated simply on having no engorged female ticks on treated animals rather than on having no larvae successfully infesting treated animals, as is required in the CFTEP. Therefore, the persistent activity claim of other investigators could be increased by up to 18 d because that is the length of time it would take larvae to reach the engorged stage of development. A study conducted with the traditional 1% formulation of doramectin against *R.* (*B.*) *microplus* at 200 μg/kg provided similar results (98% control) to this study at 14 d post-treatment, but at 28 d control was substantially lower (44.1%) than these findings (George and Davey, 2004). Conversely, the level of control of the traditional 1% injectable formulation of ivermectin at 14 d after treatment at 200 μg/kg was only 23.3%, and by 28 d post-treatment there was no control at all (Davey et al., 2005).

Regarding potential use of the LA formulation of ivermectin in the CFTEP, these results clearly indicated that the therapeutic level of control (≥99%) against ticks on the host at the time of treatment made it a highly suitable candidate for use in the program. Likewise, the material was highly effective (>99%) against ticks infested on animals within the first 14 d after treatment. Nevertheless, the persistent efficacy demonstrated in this study fell far short of the 75-d prevention against re-infestation claimed on the label. Regulations used in the CFTEP at present require all cattle (100%) held in infested premises to be dipped every 14 d, and an additional 3 d period is allowed in the event of inclement weather or difficulty in gathering all cattle (17 d total), before the herd is declared "delinquent," at which time the quarantine period is started over. The reason for this treatment interval is that coumaphos has a very short residual activity, thus dipping at this interval is the only means of ensuring that no ticks can complete their parasitic development period because ticks are unable to complete development in <18 d, as mentioned above. Thus, with regard to the LA formulation, if the 17 d period were added to the 14 d post-treatment interval, the only interval that provided the 99% level of control, it means that cattle could be treated at intervals of 31 d without risk of having viable ticks detach from infested animals. Consequently, the use of LA ivermectin in the CFTEP would reduce the number of required treatments and gathering costs by half, as compared to the presently required 14 d treatment interval.

Even though use of this LA endectocide formulation in the CFTEP could potentially reduce treatment and gathering costs by half, there are factors that could adversely impact the use of the material. First, because of the highly lipophilic nature of ivermectin the material is extensively distributed from the bloodstream to different tissues, and

distribution into adipose tissue may act as a drug reservoir thereby contributing to its long residence time (Lifschitz et al., 1999, 2000). Therefore, the repeated treatment of cattle at rather short intervals (31 d) could result in unacceptable levels of ivermectin in animal tissues. In fact, the label for the material used in the study stated that animals treated with the material could not be slaughtered for human consumption for a period of 122 d after the last treatment. Thus, the incentive for producers to maintain animals on an infested premise resulting from 50% fewer treatments would likely be offset by the fact that treated animals could not be marketed for 122 d after the last treatment. The second factor that should be considered prior to recommending the use of LA ivermectin is that repeated treatments over a long period of time, as would be necessary in the CFTEP, could increase selection pressure on off-target organisms, such as helminthes or other parasites, thereby leading to the emergence and development of endectocide resistance.

Acknowledgements

The authors thank Dr. Robert J. Miller for his assistance in obtaining the product material used in the study. The authors also thank Michael Moses for his assistance in executing the protocol for this study and collection and tabulation of the data, and Cesario Agado, James Hellums, Ruben Ramirez, Homero Vasquez, Joseph Sparks, and George Gonzalez for their assistance in the handling and care of the animals used in this study.

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